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Frequency of rs731236, rs7975232 and rs1544410 single nucleotide polymorphisms of Vitamin-D Receptor (VDR) gene among the Kazakh ethnic group

In the article the pilot study results of the genotypes and individual allele's frequency of single nucleotide polymorphisms (SNPs) rs731236, rs7975232 and rs1544410 in vitamin D receptor (VDR) gene were presented. The participants were representatives of the Kazakh ethnic group. The SNPs were determined by real-time polymerase chain reaction using the amplification-refractory mutation system (ARMS) technology. The relevance of the study is that rs731236, rs7975232 and rs1544410 contribute to the regulation of VDR gene expression. This affects the synthesis of the VDR protein and its activity as a transcription factor. VDR regulates various metabolic pathways, including the immune response to infectious diseases. In rs731236, the A allele (68.9 %) prevailed over the G (31.1 %). The AA, AG and GG genotypes were 52.1 %, 33.6 % and 14.3 %, respectively. For rs7975232, there were no significant differences in the alleles A and C (42.4 % vs. 57.6 %). There was a predominance of AC and CC genotypes (39.5 % and 37.8 %, respectively), over AA (22.7 %). Allele A of rs1544410 was not found in the Kazakh population. The allele C was 65.6 %, T — 25.6 %, and G — 8.8 %. The frequencies of the CC and CT genotypes were similar (40.3 % vs. 39.5 %). It is possible to study the influence of these SNPs on COVID-19 susceptibility among Kazakhs.

Keywords: rs731236, rs7975232, rs1544410,3'UTR polymorphism, single nucleotide polymorphism, vitamin D receptor (VDR) gene, genotype frequency, allele frequency, Kazakh ethnic group.

Introduction

Scientific databases contain many research results on the variability of vitamin D functioning in the human body. Recently, special attention has been paid to the ability of vitamin D to influence gene expression. This is due to the functioning of $1,25(OH)_2D$ which is the hormonally active form of vitamin D and a ligand for the nuclear vitamin D receptor (VDR), which is present in many tissues [1].

The active form of $1,25(OH)_2D$ binds to VDR and is capable of heterodimerizing with the retinoid X receptor (RXR). This complex can regulate the expression of up to 2000 target genes promoters of which contain specific vitamin D response elements (VDREs) [1-2]. VDR is known to be present in both T and B immune cells and regulates various metabolic pathways, including those involved in the immune response to infectious diseases and cancer [3].

At the same time, the expression level and activity of VDR are influenced by the structural features of the VDR gene located on chromosome 12q13.1. This gene has high genetic variability and more than 470 single nucleotide polymorphisms (SNPs) have been identified [4]. This article will discuss three SNPs of the VDR gene: rs731236 (TaqI), rs7975232 (ApaI) and rs1544410 (BsmI), which are in strong linkage disequilibrium with each other in the 3' untranslated region. Therefore, quite often they are combined into 3'UTR polymorphism [5-6]. These genetic changes are involved in regulating the VDR gene expression level by reducing or increasing mRNA stability [7].

Many studies of the frequency of rs731236, rs1544410 and rs7975232 genotypes and alleles have been conducted in various ethnic groups, both among healthy and in association with infectious diseases, mainly bronchopulmonary [4–6].

In this study, the frequency of rs731236, rs7975232 and rs1544410 in the VDR gene among the Kazakh ethnic group representatives was studied for the first time.

Experimental

Biological samples (blood) for the pilot study were obtained from 119 individuals of the Kazakh ethnic group of both sexes over 18 years old, residing in Karaganda city and Karaganda region. The average age was 43 years (range: 18–77). Among the participants there were 39 men and 80 women. The selection of Kazakh participants for the study was based on a questionnaire. The study was conducted in accordance with

the recommendations of the Helsinki Declaration and approved by the Local Bioethics Committee Non-commercial joint-stock company "Karaganda Medical University" (protocol No. 2, dated 11 October 2022). All participants gave written informed consent.

Blood was withdrawn into an EDTA tube. The extraction of DNA from the blood samples was performed by "RIBO-prep" (Amplisens, Russia). The SNPs were genotyped by real-time polymerase chain reaction (PCR) with forward and reverse primers (Lumiprobe, Russia) based on the amplification-refractory mutation system (ARMS) technology. Real-Time PCR DTlite (DNA Technology, Russia) was used to test the samples. Amplification conditions were selected experimentally: 3 min at 94 °C; 15 s at 94 °C and 62 °C for 30 s (×40 cycles). The sequences of primers are shown in Table.

Table

The primers sequences for rs731236, rs7975232 and rs1544410 genotyping

SNP	Alleles	Primer sequence
rs731236	A	FIP 5'-CGGTCCTGGATGGCCGCA-3'
	G	RIP5'-CAGGACGCCGCGCTGCTC-3'
		FOP 5'-TTGGCATAGAGCAGGTGGCTGCC-3'
		ROP 5'-CCCAGCTGAGAGCTCCTGTGCCTT-3'
rs7975232	A	FIP 5'-CACAGGAGCTCTCAGCTGGACA-3'
	C	RIP5'-TGGTGGGATTGAGCAGTGAAGG-3'
		FOP 5'-CCTGGATGGCCTCAATCAGC-3'
		ROP 5'-GTCATAGAGGGGTGGCCTAGGG-3'
rs1544410	C	FIP 5'-CAGAGCCTGAGTATTGGGAACGC-3'
	A	RIP 5'-GGGGCCACAGACAGGCCTACT-3'
		FOP 5'-TTTTGTACCCTGCCCGCAAGA-3'
		ROP 5'-TGTGCAGGCGATTCGTAGGG-3'
	C	FIP 5'-AGCAGAGCCTGAGTATTGGGAAAGC-3'
	G	RIP 5'-GGCCACAGACAGGCCTCCC-3'
		FOP 5'-AAGTTTTGTACCCTGCCCGCAAG-3'
		ROP 5'-GTGCAGGCGATTCGTAGGGG-3'
	T	FIP 5'-GCAGAGCCTGAGTATTGGGAAGGT-3'
	C	RIP 5'-GGCCACAGACAGGCCTTCG-3'
		FOP 5'-AAGTTTTGTACCCTGCCCGCAA-3'
		ROP 5'-TGTGCAGGCGATTCGTAGGG-3'

The chi-square test (χ^2 test) was used to compare categorical data and $p < 0.05$ was considered statistically significant. All SNPs were tested for Hardy–Weinberg equilibrium using chi-squared test. The statistical analyses were carried out by the GraphPad Prism 8.0 program (Graph-Pad Software, CA, USA).

Results and Discussion

The frequency of 3'UTR polymorphism in VDR gene was identified among Kazakhs. The observed rs731236, rs7975232 and rs1544410 SNPs were consistent with Hardy–Weinberg equilibrium with $p > 0.05$ ($p = 0.0628$, $p = 0.1125$, $p = 0.5381$ respectively).

The data on the allelic composition of rs731236 are inconclusive. According to some authors, this polymorphism represents an allelic substitution T>C, leading to silent mutations in the corresponding codons [5, 7]. At the same time, dbSNP (Current build 156, released September 21, 2022) characterizes rs731236 as a three-allelic polymorphism with predominant alleles A and G [8]. The data on the frequency of the rs731236 genotypes and alleles among the Kazakh population are shown in Figure 1.

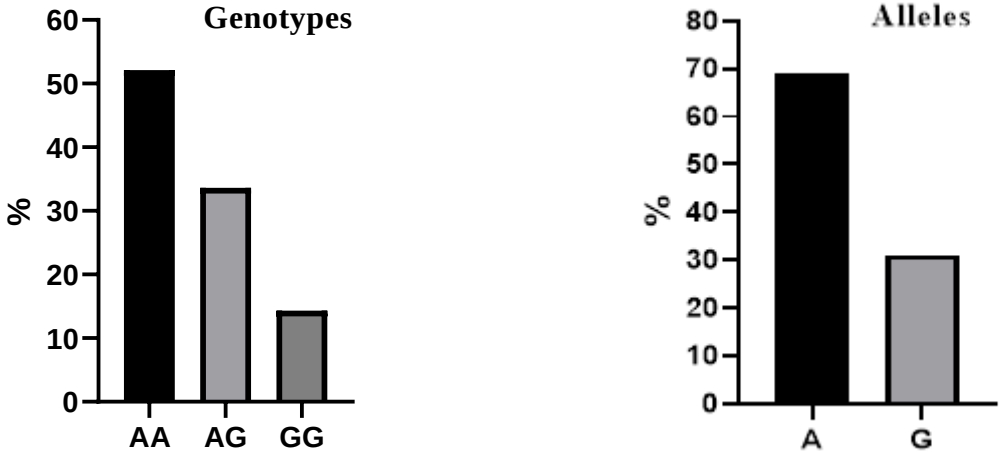

Figure 1. The rs731236 genotypes and alleles frequency in the Kazakh population

The A allele of rs731236 is more than twice as common as the G allele (68.9 % vs. 31.1 %) in the study sample. The homozygous genotype AA rs731236 also predominated and was found in half of the samples (52.1 %). The GG genotype was the rarest (14.3 %), and AG heterozygotes were found in a third of cases (33.6 %). A comparison of the rs731236 allele frequency with other sources was made (Fig. 2).

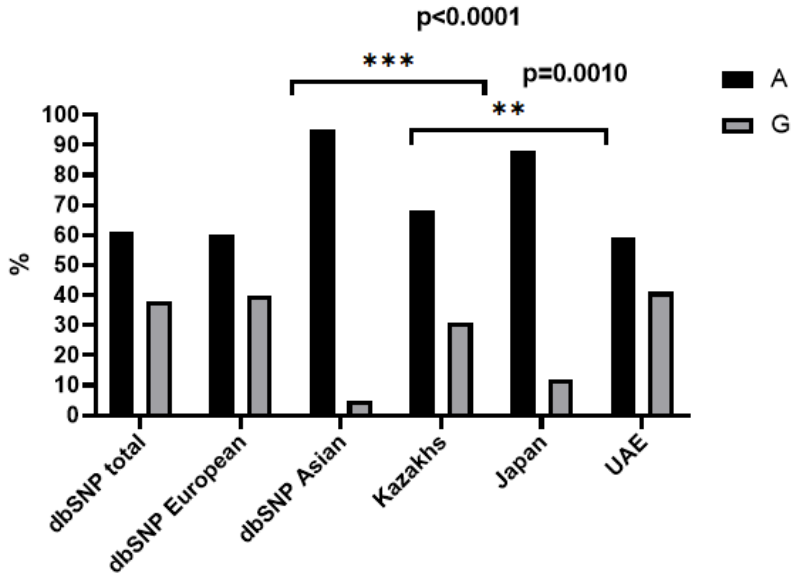

Figure 2. Comparison of allele frequency data for rs731236

The data obtained show a slight predominance of A allele over G allele compared to dbSNP total data (61.5 % and 38.5 % for alleles A and G, respectively), but this difference was not statistically significant ($p > 0.05$) [8]. The results of the present study were very similar to the dbSNP European (A = 60.2 %, G = 39.8 %, $p > 0.05$) [8]. Also, similar values were found in the UAE (A = 59.2 %, G = 40.8 %, $p > 0.05$) [4]. The dbSNP Asian demonstrates the predominance of allele A over G (95 % vs. 5 %) [8], which is statistically different from the data of the Kazakh population ($p < 0.0001$). Some published data from Japan (Tokitan et al.), where A = 88 %, G = 12 % [11] confirms these results ($p = 0.0010$).

The allele frequency of Kazakhs was similar to the UAE and statistically different from Japan. However, the situation was different regarding the distribution of genotypes. In Japan and Kazakhstan, AA homozygotes predominated (52.1 % and 77 %, respectively) [11], while in the UAE heterozygotes AG were more often found (42.4 %) [4]. Common to all three populations was the lowest frequency of the GG genotype (14.3 % — Kazakhs, 19.6 % — UAE, 1 % — Japan) [4, 11].

Single nucleotide polymorphism rs7975232 of the VDR gene includes two alternative alleles A and C, which is consistent with both dbSNP (Current build 156, released September 21, 2022) [9] and most publications [5, 12]. The ratio of alleles in different populations may vary. Data on the frequency of the rs7975232 in the Kazakh population are depicted in Figure 3.

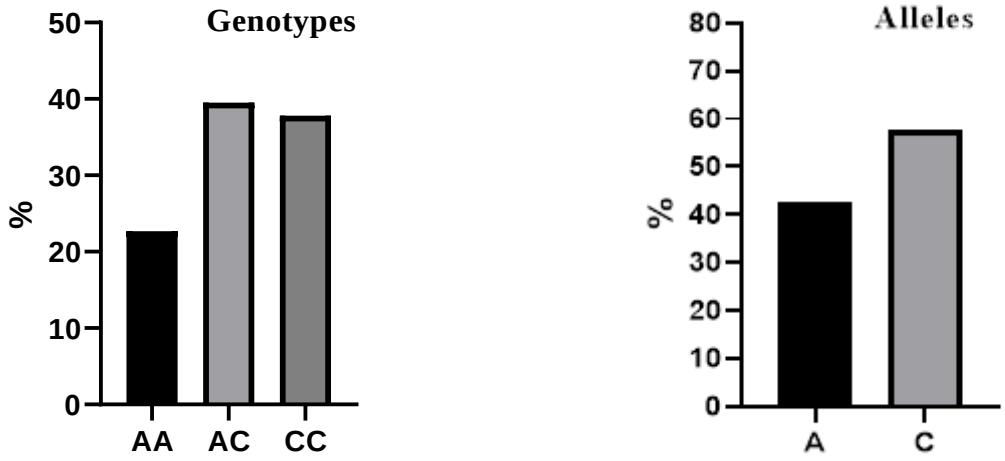

Figure 3. The rs7975232 genotypes and alleles frequency in the Kazakh population

The frequency of alleles A and C among the Kazakh ethnic group differs slightly (42.4 % vs. 57.6 %). However, heterozygous genotype AC and homozygous CC are found almost equally (39.5 % and 37.8 %, respectively), and homozygous AA are less common (22.7 %). Thus, for rs7975232 there is a predominance of heterozygotes over homozygotes ones. Allele frequencies of rs7975232 vary in different sources (Fig. 4).

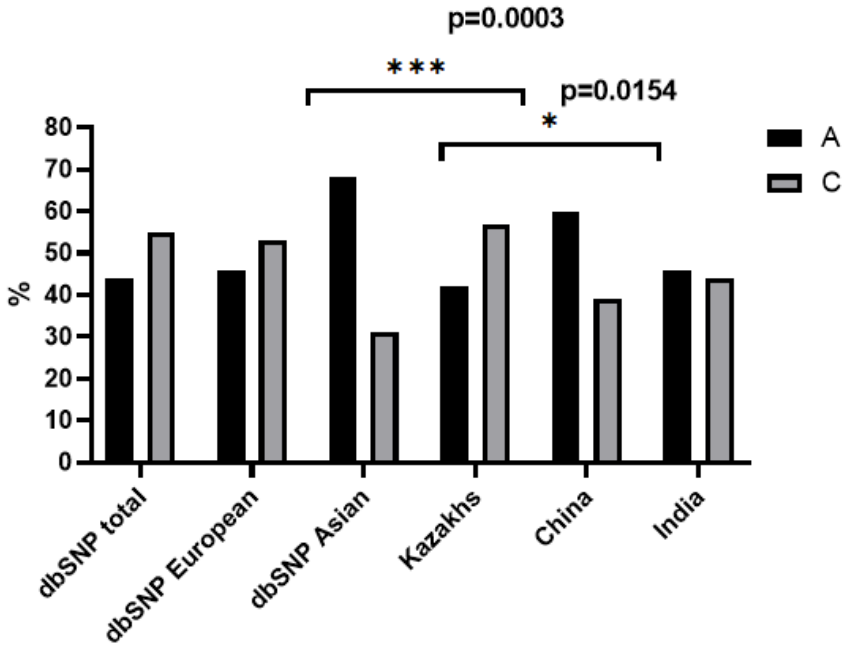

Figure 4. Comparison of allele frequency data for rs7975232

The allele frequency of rs7975232 in the Kazakh population maintains a similar trend with rs731236. Also, the obtained data are consistent with dbSNP total (A = 44.5 %, C = 55.5 %) and European (A = 46.3 % and C = 53.7 %) [9], as well as with India (A = 46 %, C = 44 %) ($p > 0.05$) [12]. Statistically significant differences were found to a greater extent with dbSNP Asian, where A = 68.7 % and C = 31.3 % ($p = 0.0003$) [10] and to a lesser extent with China (60.5 % vs. 39.5 %, respectively) ($p = 0.0154$) [12].

When comparing the frequency of genotypes in the populations of Kazakhstan, India and China, the most common were heterozygotes AC (39.5 %, 45.4 % and 55 %, respectively). Homozygotes AA (22.7 %) were the rarest only among Kazakhs. In India and China, the frequency of AA was similar (33 %), and CC homozygotes were the least common (21.6 % and 12 %, respectively) [12].

The latest single-nucleotide polymorphism within the 3'UTR, rs1544410, is reported to exhibit a tetra-allelic nature according to dbSNP data (Current build 156, released September 21, 2022) [10]. The obtained results of rs1544410 genotyping in the Kazakh population are depicted in Figure 5.

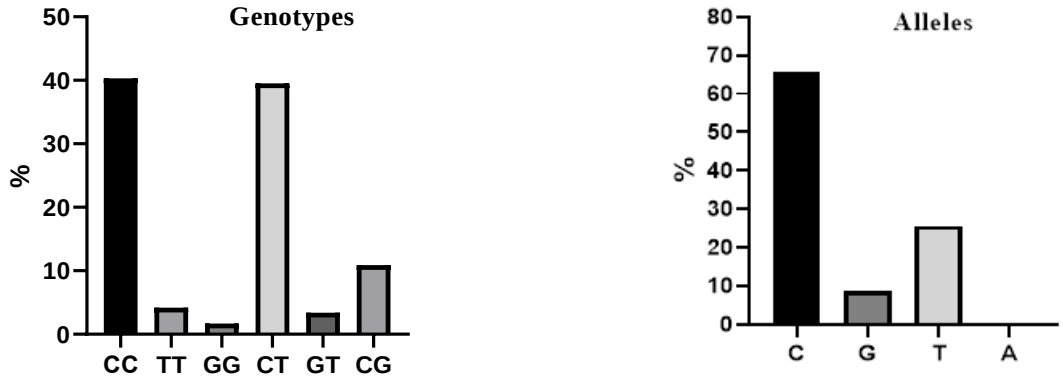

Figure 5. The rs1544410 genotypes and alleles frequency in the Kazakh population

The allele A of rs1544410 was not detected among the Kazakhs. In the majority of samples, the allele C was prevalent (65.6 %), while the allele T was significantly less common (25.6 %). The G allele (8.8 %) was rare and was mainly detected in the form of heterozygotes, such as GC (10.9 %) and GT (3.4 %). Homozygotes GG were found in two samples out of the total number (n=119). Interestingly, the allele T was rarely encountered in a homozygous state (TT frequency = 4.2 %), while the heterozygous genotype CT was detected at a frequency similar to homozygous CC (39.5 % vs. 40.3 %).

In spite of the fact that, according to dbSNP data, rs1544410 encompasses four alleles [10], scientific publications typically focus on the simultaneous examination of the frequency of only two alleles in various combinations: A>G [5,7], A>T [12], which complicates comparative analysis.

The frequency of rs1544410 was compared only with dbSNP data (Fig. 6) for the C and T alleles (as a percentage of total occurrence; *n* = 217). This approach was used because publications mainly determine the frequency of alleles A and G. But allele A was not identified in the Kazakh population, and the frequency of allele G was rare.

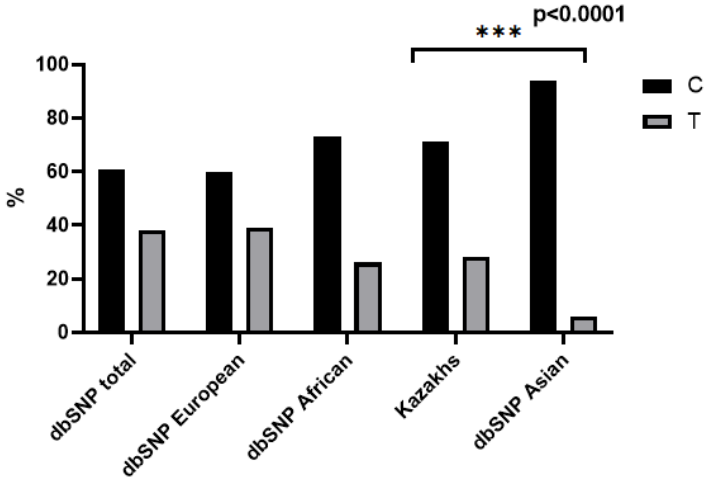

Figure 6. Comparison of allele frequency data for rs1544410

The allelic frequency coincidence was observed between alleles C and T rs1544410 with the total(C = 61.2 %, T = 38.8 %), European (C = 60.1 %, T = 39.9 %), and African (C = 73.7 %, T = 26.3 %) dbSNP data ($p > 0.05$). Additionally, a significant statistical difference was found with dbSNP Asian data, where C = 94 %, T = 6 % ($p<0.0001$) [10].

Thus, the frequency of the main alleles of all three SNPs included in the VDR gene 3'UTR (rs731236, rs7975232 and rs1544410) in the Kazakh population is statistically similar to the total and European dbSNP data, while all of them showed statistically significant differences with dbSNP data for the Asian population [8–10]. This may be due to the linkage of rs731236, rs7975232 and rs1544410 to each other [5-6].

Conclusion

In this study, 119 biological samples were genotyped to determine the allele and genotype frequency of SNPs rs731236, rs7975232, and rs1544410 among individuals of the Kazakh ethnic group. All SNPs are in a non-equilibrium linkage with each other and aggregate into a 3'UTR polymorphism. In the case of rs731236, the most frequently encountered allele was A (68.9 %), while allele G was found in 31.1 % of cases. Among the genotypes, AA predominated (52.1 %), the number of heterozygotes AG constituted 33.6 %, and the homozygous GG was less common (14.3 %). For rs7975232, significant differences in the allele frequency of A and C (42.4 % vs. 57.6 %) was not detected; however, the distribution of genotypes in the population revealed a prevalence of AC and CC (39.5 % and 37.8 %, respectively) over AA (22.7 %). The rs1544410 is four-allelic; however, allele A was not detected in the Kazakh population, and the frequency of allele G was low (8.8 %). The major allele was C (65.6 %), and the T allele was found only in 25.6 %. However, the frequency of CC and CT genotypes was similar (40.3 % vs. 39.5 %). The obtained results are of interest as a general population study and serve as a comparative dataset. Additionally, the potential role of these SNPs on susceptibility to COVID-19 among Kazakhs is planned to be investigated.

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Қазақ этникалық тобының өкілдері арасында D дәрумені рецепторы генінің (VDR) rs731236, rs7975232 және rs1544410 бірнуклеотидті полиморфизмдерінің кездесу жиілігі

Мақалада D (VDR) дәрумені рецепторларының геніндегі rs731236, rs7975232 және rs1544410 бірнуклеотидті полиморфизмдердің (SNP) генотиптері мен жеке аллельдерінің жиілігін зерттеудің пилоттық нәтижелері келтірілген. Зерттеу қазақ этникалық тобының өкілдерінің қатысуымен жүргізілді. SNP амплификацияға төзімді мутация жүйесінің технологиясын (ARMS) қолдана отырып, нақты уақыт режимінде полимеразды тізбекті реакция әдісімен анықталды. Зерттеудің өзектілігі rs731236, rs7975232 және rs1544410 D витаминінің рецепторлық ақуыз синтезіне және оның транскрипция факторы ретіндегі белсенділігіне әсер ететін VDR генінің экспрессия деңгейін реттеуге қатысатындығына байланысты. VDR әртүрлі метаболикалық жолдарды, соның ішінде жұқпалы ауруларға иммундық жауапты реттейді. Rs731236-да A аллель жиілігі (68,9 %) G аллельден басым болды (31,1 %). AA, AG және GG генотиптері сәйкесінше 52,1 %, 33,6 % және 14,3 % құрады. Rs7975232 үшін A және C аллельдерінде айтарлықтай айырмашылықтар анықталмады (57,6 % қарсы 42,4 %), ал популяциядағы генотиптердің таралуы AA-ға (22,7%) қарағанда AC және CC (тиісінше 39,5% және 37,8%) басымдылығын көрсетті. A аллелі rs1544410 қазақ популяциясында табылған жоқ. C аллелі 65,6 %, T — 25,6 % және G — 8,8 % құрады. CC және CT генотиптерінің жиілігі ұқсас болды (40,3 % қарсы 39,5 %). SNP деректерін ағзаның COVID-19 сезімталдығына әсер ететін ықтимал факторлар ретінде зерттеу мүмкіндігі көрсетілген.

Кілт сөздер: rs731236, rs7975232, rs1544410, 3'UTR полиморфизмі, бірнуклеотидті полиморфизм, D дәрумені рецепторларының гені (VDR), генотип жиілігі, аллель жиілігі, қазақ этникалық тобы.

В.В. Протас, Г.П. Погосян, К.Г. Ли

Частота встречаемости однонуклеотидных полиморфизмов rs731236, rs7975232 и rs1544410 гена рецептора витамина D (VDR) среди представителей казахской этнической группы

В статье представлены результаты пилотного исследования по изучению частоты генотипов и отдельных аллелей однонуклеотидных полиморфизмов (SNP) rs731236, rs7975232 и rs1544410 в гене рецептора витамина D (VDR). Исследование проводилось с участием представителей казахской этнической группы. SNP определяли с помощью метода полимеразной цепной реакции в режиме реального времени с использованием технологии системы устойчивых к амплификации мутаций (ARMS). Актуальность исследования обусловлена тем, что rs731236, rs7975232 и rs1544410 участвуют в регуляции уровня экспрессии гена VDR, что влияет на синтез белка-рецептора витамина D и его активность как транскрипционного фактора. VDR регулирует различные метаболические пути, включая иммунный ответ на инфекционные заболевания. В rs731236 частота аллели A (68,9 %) преобладала над G (31,1 %). Генотипы AA, AG и GG составили 52,1 %, 33,6 % и 14,3 % соответственно. Для rs7975232 не было выявлено достоверных различий по аллелям A и C (42,4 % против 57,6 %), при этом распределение генотипов в популяции показало преобладание AC и CC (39,5 % и 37,8 % соответственно) над AA (22,7 %). Аллель A rs1544410 не был обнаружен в казахской популяции. Аллель C составил 65,6 %, T — 25,6 % и G — 8,8 %. Частоты генотипов CC и CT были сходными (40,3 % против 39,5 %). Показана возможность изучения данных SNP как потенциальных факторов, влияющих на восприимчивость организма к COVID–19.

Ключевые слова: rs731236, rs7975232, rs1544410, полиморфизм 3'UTR, однонуклеотидный полиморфизм, ген рецептора витамина D (VDR), частота генотипа, частота аллеля, казахская этническая группа.

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